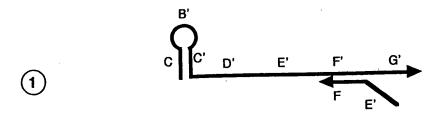


FIGURE 1

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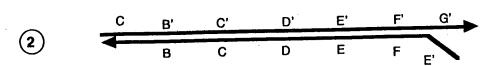
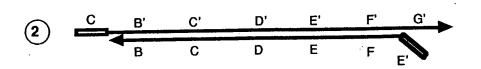
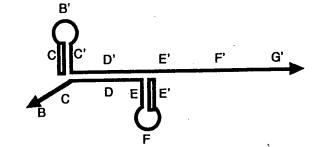


FIGURE 3









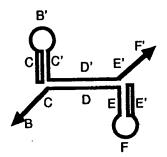
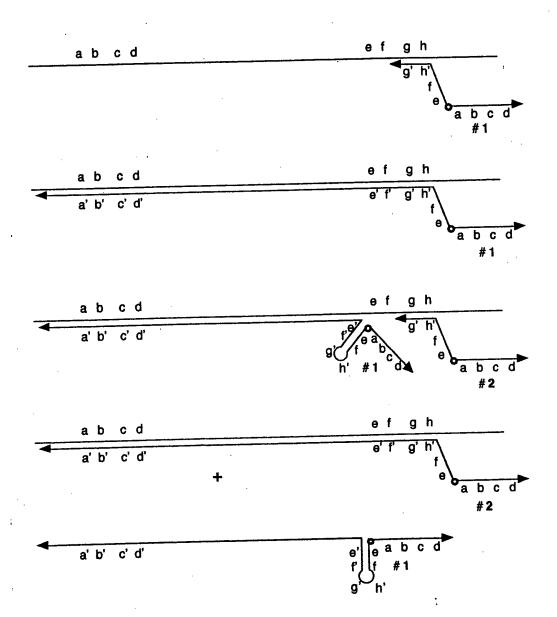
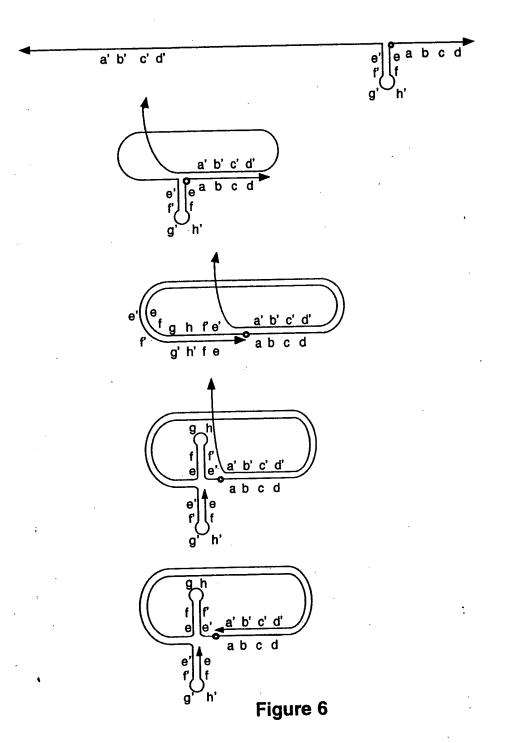


FIGURE 4



 $\mathcal{J}_{\mu}$  :

Figure 5



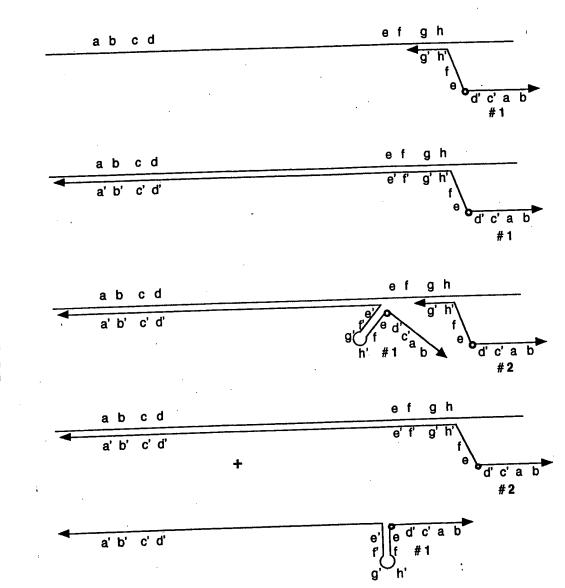


Figure 7

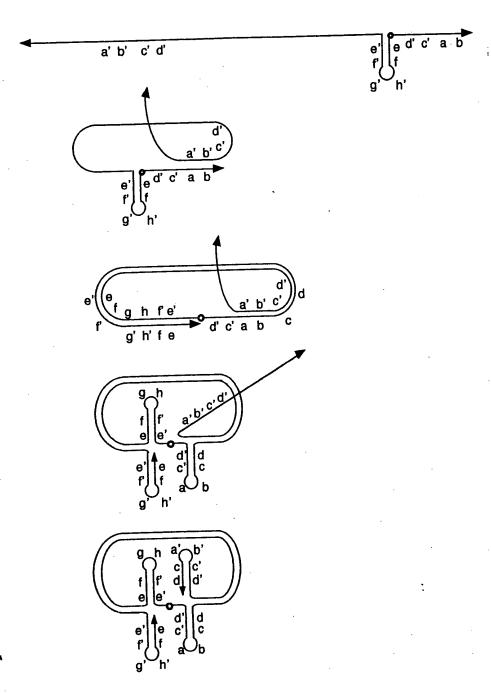


Figure 8

a' b'

6

a b

 $\hat{u}_{\mathbf{y}}^{\mathbf{y}^{*}}\hat{j}$ 

FIGURE 9

c' d'

b a

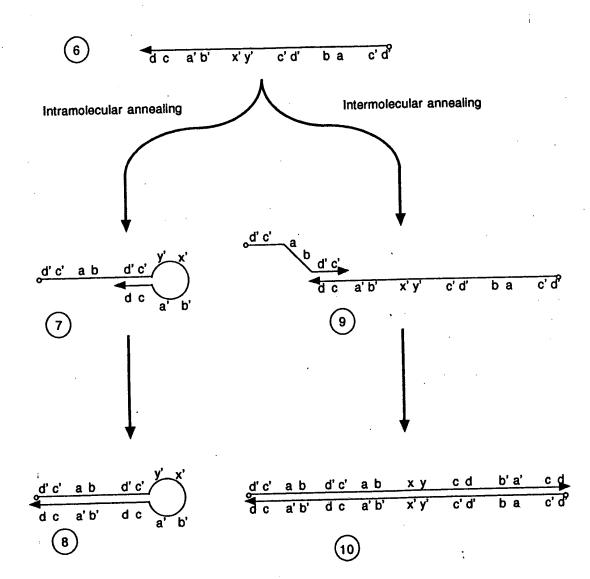


FIGURE 10

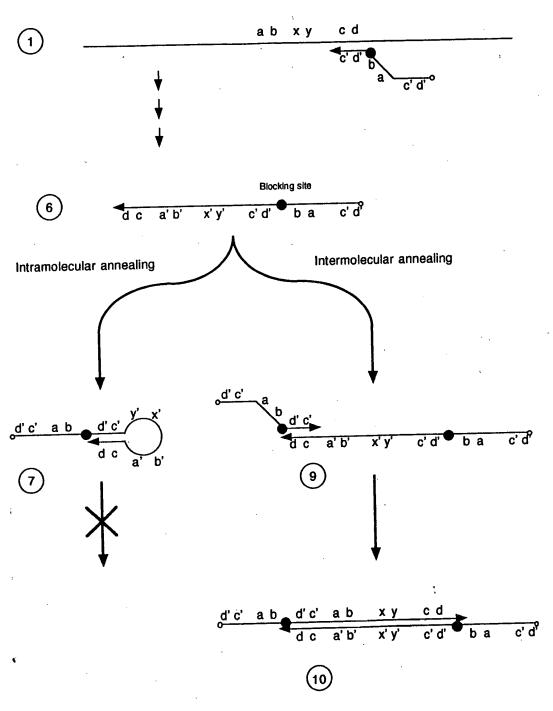


FIGURE 11

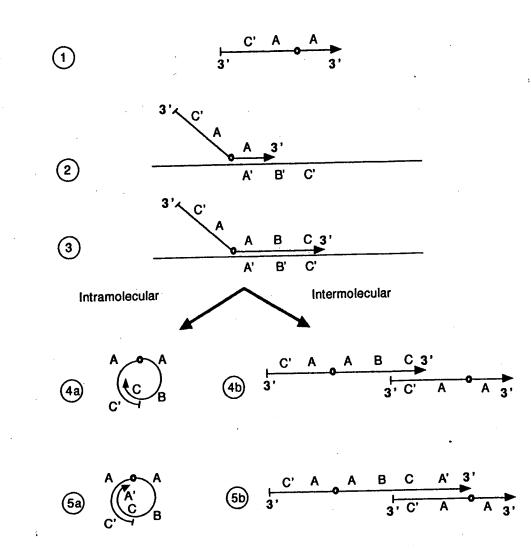


FIGURE 12

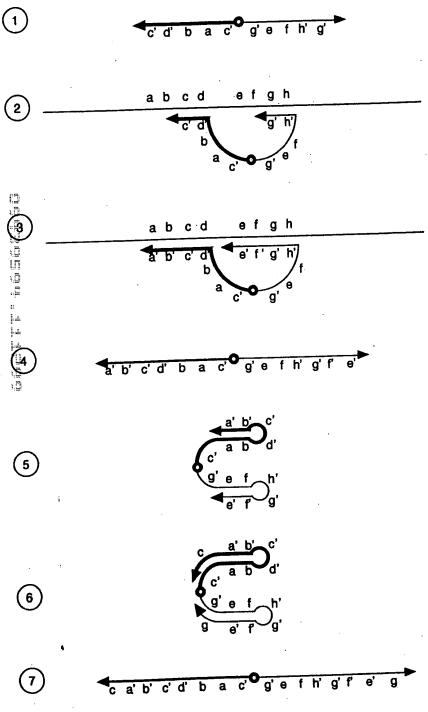


FIGURE 13

FIGURE 14

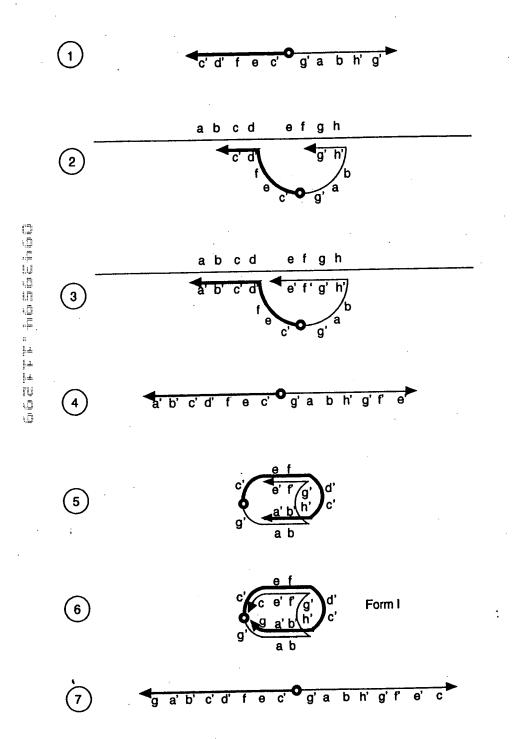
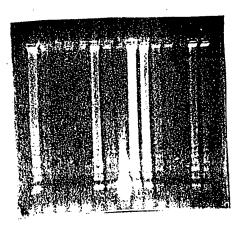


FIGURE 15

Form II

FIGURE 16

Form III





30 Minutes Incubation

180 Minutes Incubation

- 53°C, 10<sup>-2</sup> dilution 53°C, 10<sup>-3</sup> dilution
- 53°C, 10<sup>-4</sup> dilution 53°C, 10<sup>-6</sup> dilution 53°C, No Target

- 53°C, 10°2 dilution, FC/LRC 53°C, 10°2 dilution, LFC/RC
- Msp I Marker 8
- 9 63°C, 10°2 dilution 10 63°C, 10°3 dilution 11 63°C, 10°4 dilution 12 63°C, 10°5 dilution 13 63°C, No Target

- 14 63°C, 10°2 dilution, FC/LRC 15 63°C, 10°2 dilution, LFC/RC

FIGURE 17

# A) Gel assay

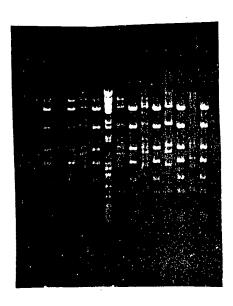
Top = Isothermal Amplification Bottom = PCR Amplification

- 1
- Msp I Marker
  1 x 10<sup>6</sup> target
  1 x 10<sup>4</sup> target
  1 x 10<sup>2</sup> target
  No target 2
- 3
- 4
- 5



## B) Plate Assay

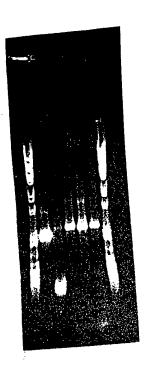
10 <sup>6</sup> target	10 <sup>4</sup> target	10 <sup>2</sup> target	No target
1.702	1.594	0.376	0.085



Carboxy-U, Klenow, 37°C, NEB #2 Normal T, Klenow, 37°C, NEB #2 2٠ Carboxy-U, Klenow, 37°C, Buffer #2A 3 Normal T, Klenow, 37°C, Buffer #2A 4 Carboxy-U, Klenow, 55°C, NEB #2 5 Normal T, Klenow, 55°C, NEB #2 6 7 Msp I Marker Carboxy-U, Taq, 55°C, NEB #2 8 Normal T, Taq, 55°C, NEB #2 9 Carboxy-U, Taq, 65°C, Buffer #2M 10 Normal T, Taq, 65°C, Buffer #2M 11 Carboxy-U, Bst 65°C, Thermopol Buffer 12 Normal T, Bst, 65°C, Thermopol Buffer 13 Carboxy-U, Taq, 65°C, Buffer #2A 14 Normal T, Taq, 65°C, Buffer #2A 15

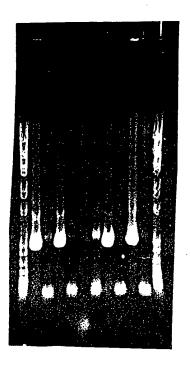
FIGURE 20

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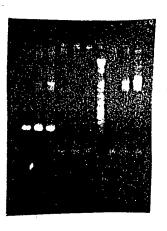


- Msp I/Bst E II marker Normal T, 1 mM MgCl<sub>2</sub> Carboxy U, 2 mM MgCl<sub>2</sub> Carboxy U, 3 mM MgCl<sub>2</sub> 1. 2. 3. 4. 5. 6.
- Carboxy U, 4 mM MgCl<sub>2</sub> Carboxy U, 5 mM MgCl<sub>2</sub> Msp I/Bst E II marker
- 7.

FIGURE 21



- 1. Msp I/Bst E II marker
- 2. Normal T, Taq
- 3. Carboxy U, Taq
- 4. Normal T, Tfl
- 5. Carboxy U, Tfl
- 6. Normal T, Tth
- 7. Carboxy U, Tth
- 8. Normal T, Amplitherm
- 9. Carboxy U, Amplitherm
- 10. Normal T, Replitherm
- 11. Carboxy U, Replitherm
- 12. Msp I/Bst E II marker



Taq, 2mM MgCl<sub>2</sub>
 Taq, 4mM MgCl<sub>2</sub>
 Taq, 6mM MgCl<sub>2</sub>
 Tfl, 2mM MgCl<sub>2</sub>
 Tfl, 4mM MgCl<sub>2</sub>
 Tfl, 6mM MgCl<sub>2</sub>
 Msp I marker
 Tfl/Enh, 2mM MgCl<sub>2</sub>
 Tfl/Enh, 4mM MgCl<sub>2</sub>
 Tfl/Enh, 6mM MgCl<sub>2</sub>



ir.

- 1. Tth/Enh, 4mM MgCl<sub>2</sub>
- 2. Tth/Enh, 6mM MgCl<sub>2</sub>
- 3. Tth/Enh, 8mM MgCl<sub>2</sub>
- 4. Msp I/BspE1 marker
- 5. Amplitherm/ Enh, 4mM MgCl<sub>2</sub>
- 6. Amplitherm/ Enh, 6mM MgCl<sub>2</sub>
- 7. Amplitherm/ Enh, 8mM MgCl<sub>2</sub>
- 8. Msp I/BspE1 marker
- 9. Replitherm/ Enh, 4mM MgCl<sub>2</sub>
- 10. Replitherm/ Enh, 6mM MgCl<sub>2</sub>
- 11. Replitherm/ Enh, 8mM MgCl<sub>2</sub>



....

- 1. Msp I marker
- 2. 0.3X enhancer
- 3. Control
- 4. deaza G
- 5. Gene 32
- 6. 10% DMS0
- 7. 3X polymerase

5'-TGC GCT GCT AAC AAA GCC CGA AAG GAA G------GCT GAA AGG AGG AAC TAT ATG GCG TCA TAC GAT ATG AAC GTT-3'
3'-ACG CCA CCA TTG TTT CGG GCT TTC CTT C------CGA CTT TCC TCC TTG ATA TAC GCG AGT ATG CTA TAC TTG CAA-5'

5'-AAT CTA GA GCT AAC AAA GCC CGA AAG GAA G-3'

3'-CGA CIT TCC TCC TIG ATA TA GAC GTC TT-5'

TS-14

TS-21

5'-TGC GCT GCT AAC AAA GCC CGA AAG GAA G-3'

5'-ACC CGC GCT GCT AAC AAA GCC CGA AAG GAA G-3'

TS-22

TS-23

3'-CGA CIT TCC TCC TTG ATA TAC GCG AGT-5'

FIGURE 26

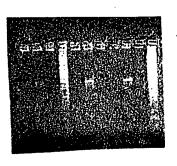
TS-24

3'-G ATA TAC GCG AGT ATG CTA TAC TTG CAA-5'



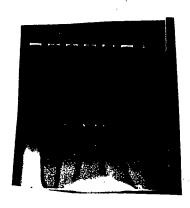
```
1.
        Msp I marker
     TS13 + TS14
2.
3.
     TS13 + TS23
     TS13 + TS24
4.
     TS21 + TS14
5.
     TS21 + TS23
6.
     TS21 + TS24
7.
8.
     TS22 + TS14
     TS22 + TS23
9.
     TS22 + TS24
10.
        Msp I marker
11.
     TS13 + TS14 (different lot of C-U)
12.
     TS13 + TS14 (allylamine dUTP)
13
      TS13 + TS14 (normal dTTP)
14.
```

FIGURE 27

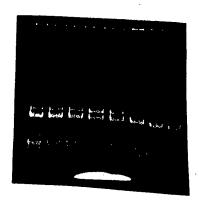


- TS13 + TS14 1.
- 2. TS13 + TS23
- 3. TS13 + TS24
- Msp I marker 4.
- TS21 + TS14
- 5. 6. 7. TS21 + TS23 TS21 + TS24
- 8. TS22 + TS14
- 9.
- 10.
- TS22 + TS23 TS22 + TS24 Msp I marker 11.

### Fluorescent detection



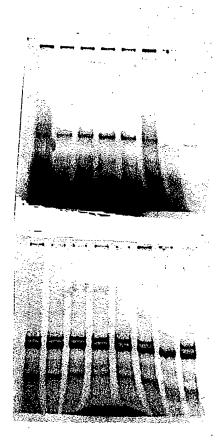
### Ethidium Bromide fluorescence



- 1 x TAPS, pH 9.2 2 x TAPS, pH 9.2 2
- 3 x TAPS, pH 9.2 3 x TAPS, pH 9.7 3 x TAPS, pH 9.2 3
- 5
- 3 x TAPS, pH 8.6 6
- 7 No enzyme control
- 8 Fluorescein 12-ddUTP control

### Fluorescent detection

**Ethidium Bromide fluorescence** 



- 1 1 x TAPS, pH 9.2
- 2 2 x TAPS, pH 9.2
- 3 3 x TAPS, pH 9.2
- 4 3 x TAPS, pH 9.7
- 5 3 x TAPS, pH 9.2
- 6 3 x TAPS, pH 8.6
- 7 No enzyme control
- 8 Fluorescein 12-ddUTP control